Synthesis and anti-inflammatory effects of new piperazine and ethanolamine derivatives of H_1-antihistaminic drugs

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Abstract: In addition to their antihistamine effects, H_1-receptor antagonists possess pharmacological properties that are not uniformly distributed among this class of drugs, such as anti-inflammatory, anti-allergic and antiplatelet activities. In this paper, Cyclizine (1-benzhydryl-4-methyl-piperazine, **I**), bromodiphenhydramine (2-[(4-bromophenyl)-phenylmethoxy]-*N*, *N*-dimethylethanamine, **II)** and some of their new piperazine and ethanolamine derivatives (**III-VIII**) inducing changes in substitution of phenyl and amine moieties were synthesized and their acute and chronic anti-inflammatory effects were evaluated by standard pharmacological tests. The results showed that substitution of phenyl by tolyl, anisol and cumene groups in piperazine family could remarkably decrease acute inflammation in these new drugs. Also, substitution of dimethylamine by morpholine group could not decrease this inflammation in new synthesized ethanolamine family. But the results from the cotton pellet-induced granuloma formation in rats showed that none of drugs (**I-VIII**) were effective to reduce the chronic inflammation.

Keywords: Acute and chronic anti-inflammation effects, Cyclizine, Bromo diphenhydramine, H_1-receptor antagonist, Piperazine and ethanolamine derivatives.

1. INTRODUCTION

Histamine is an intercellular chemical messenger which plays a critical role in several diverse physiological processes. Four human G-proteins coupled histamine receptor subtypes (H_{1-4}) are currently recognized to mediate various actions of the monoamine histamine. These include smooth muscle contraction, inflammatory response, gastric acid secretion, and mediation of neurotransmitter release in central nervous system [1].

Histamine has a key role in allergic inflammatory conditions, too. The inflammatory responses resulting from the liberation of histamine have long been thought to be mediated by the histamine H_1 receptor, and H_1 receptor antagonists - commonly known as antihistamines - which have been used in treatment of various allergic and inflammatory conditions due to histamine release for many years [2]. Although antihistamines belong to several different chemical classes, such as ethylene-diamines, aminoethylethers, propyl- and propenyl- arnines, phenothiazines, piperidines and piperazines, they show remarkable chemical similarities [3].

In addition to their antihistamine effects, H_1-receptor antagonists possess pharmacological properties that are not uniformly distributed among this class of drugs, such as anti-inflammatory and anti-allergic effects, antiplatelet activity, and suppressing respiratory burst of professional phagocytes [4-6]. Increasing evidence suggests that H_1-antihistamines have anti-inflammatory and immunomodulatory actions that are more extensive than it can simply be explained by H_1-receptor blockade [7,8].

The benzhydryl piperazine and aminoalkylamine are fundamental components present in the antihistamine drugs, such as Cyclizine, Cinnarazine, Oxatomide (piperazine class of H_1-receptor antagonist) and diphenhydramine, doxylamine, orphenadrine and bromazine (ethanolamine class of H_1-receptor antagonist). By changing the nitrogen substitution of piperazine and ethanolamine in the basic moiety as well as substitution of phenyl groups in molecules, the resultant derivatives possess broad pharmacological activities in central nervous system [9, 10].

In this paper, Cyclizine (1-benzhydryl-4-methyl-piperazine, CAS 82-92-8, CYC, **I**), bromodiphenhydramine (2-[(4-bromophenyl)-phenylmethoxy]-*N*,*N*-dimethylethanamine, Bromazine, CAS 1808-12-4, Br-DPH, **II**) (well known drugs in H_1-receptor antagonist family), and some of their new piperazine and ethanolamine derivatives, including:

1-ethyl-4-[(*4*-isopropylphenyl)(*4'*-methoxyphenyl)methyl]-piperazine (**III**)

1-(3,4-dichlorophenyl)-4-[(*4*-tolyl)(*4'*-methoxyphenyl)methyl]-piperazine (**IV**)

1-(3,4-dichlorophenyl)-4-[di(*p*-tolyl)methyl]-piperazine (**V**)

1-ethyl-4-[di(*p*-tolyl)methyl]-piperazine (**VI**)

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2-[(*4*-isopropylphenyl)(*4'*-methoxyphenyl)methoxy]-*N*,*N*-dimethylethanamine (**VII**)

2-[(*4*-isopropylphenyl)(*4'*-methoxyphenyl)methoxy]-ethylmorpholine (**VIII**)

Were all synthesized and the acute and their chronic anti-inflammatory activities evaluated by the known pharmacological procedures [11-13].

2. MATERIAL AND METHODS

2.1. General

1-methyl piperazine, 1-ethyl piperazine, 1-(3,4-dichlorophenyl) piperazine, 4-bromo toluene, benzaldehyde, thionyl chloride, 4-bromo benzaldehyde, 4-methyl benzaldehyde, 4-isopropyl benzaldehyde, 4-bromo anisole, acethyl bromide, dimethyl aminoethanol, 2-morpholino ethanol, magnesium turning, diethyl ether, xylene, benzene and all other chemicals were purchased from Merck chemical Co. (Darmstadt, Germany). Melting points (incorrected) were determined using a digital Electrothermal melting point apparatus (***model 9100***, ***Electrothermal Engineering Ltd***., Essex, ***UK***). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded on a Thermo Nicolet FT-IR (model ***Nexus-870***, Nicolet Instrument Corp, Madison, Wisconsin, U.S.A.) spectrometer. Mass spectra were recorded on an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmigton, USA). Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400 and were within ± 0.4% of the theoretical values. Adult male wistar rats (Pasteur Institute, Tehran, weighing 250-300g were used for pharmacological testing.

2.2. Preparations (Scheme 1-3)

2.2.1. Diphenylmethanol (Benzhydrol) (1)

This compound was prepared based on a published method [14, 15]. To a THF (50ml) solution of benzaldehyde (10.6g, 0.1mol), phenyl magnesium bromide (prepared from 15.7g bromobenzene and 2.43g of Mg in 50ml of dry ether) was added dropwise and refluxed for additional 118 hours. Then, it was poured into ice-NH_4Cl and the organic layer was separated, washed with brine, re-extracted with diethyl ether, dried over $MgSO_4$ and evaporated under vacuum. The solid compound (m.p.: 64-66° C) was obtained (Scheme **2**).

2.2.2. Chlorodiphenylmethan (Benzhydryl chloride) (2)

This compound was prepared based on a published method [14, 15]. $SOCl_2$ (8ml, 0.11mol) was added to a dichloromethane (250ml) solution of alcohol (**1**, 10.5g, 0.05mol). The mixture was refluxed for additional 210 hours and the solvent was evaporated under vacuum to obtain a brown oily compound which was used in the next step without further purification (high sensitive to light) (Scheme **2**).

2.2.3. 1-Benzhydryl-4-methyl-piperazine (Cyclizine) I

This compound was prepared based on a published method [14, 15]. 1-methyl piperazine (15g, 0.15mol) was added to an acetonitrile (100ml) solution of benzhydryl chloride (**2**, 8g, 0.04mol). The mixture was refluxed for additional 280 hours, before the solvent was removed under vacuum, extracted with diethyl ether, washed with water, re-extracted with 10% H_2SO_4, and neutralized with 10% NaOH. The organic layer was successively water-washed, dried over $MgSO_4$ and evaporated under vacuum to obtain the desired compound (m.p.: 105-108) (high sensitive to light) (Scheme **2**).The hydrochloride salt of **I** was prepared using diethyl ether and HCl (high sensitive to light).

*2.2.4. (***p****-isopropylphenyl)(***p****-anisol) methanol (3)***

This compound was prepared based on a published method [15]. To a THF (50ml) solution of *p*-isopropyl benzaldehyde (14.82g, 0.1mol), anisol magnesium bromide (prepared from 17g *p*-bromo anisol and 2.5g of Mg in 50 ml of dry ether) was added dropwise and refluxed for additional 6 days. Then, it was poured into ice-NH_4Cl and the organic layer was separated, washed with brine, re-extracted with diethyl ether, dried over $MgSO_4$ and evaporated under vacuum to obtain the oily compound (8.6g, 33.6% yield) (Scheme **2**).

*2.2.5. Chloro (***p****-isopropylphenyl)(***p****-anisoll) methan (4)***

This compound was prepared based on a published method [16]. $SOCl_2$ (8ml, 0.11mol) was added to a dichloromethane (250ml) solution of alcohol (**3**, 12.8g, 0.05mol). The mixture was refluxed for additional 210 hours and the solvent was evaporated under vacuum to obtain a brown oily compound (5.3g, 57.6% yield) which was used in the next step without further purification (high sensitive to light) (Scheme **2**).

*2.2.6. 1-ethyl-4-[(***p****-isopropylphenyl)(***p****-anisol) methyl]-piperazine (Cycl-1) III***

1-ethyl piperazine (19ml, 0.1mol) was added to an acetonitrile (100ml) solution of **4** (8g, 0.027mol). The mixture was refluxed for additional 280 hours. Then, the solvent was removed under vacuum, extracted with diethyl ether, water-washed, re-extracted with 10% H_2SO_4, and neutralized with 10% NaOH. The organic layer was successively water-washed, dried over $MgSO_4$, and evaporated under vacuum to obtain the oily compound (2.1g, 31% yield) (high sensitive to light) (Scheme **2**). The hydrochloride salt of **III** (oily compound) was prepared using diethyl ether and HCl (highly sensitive to light).

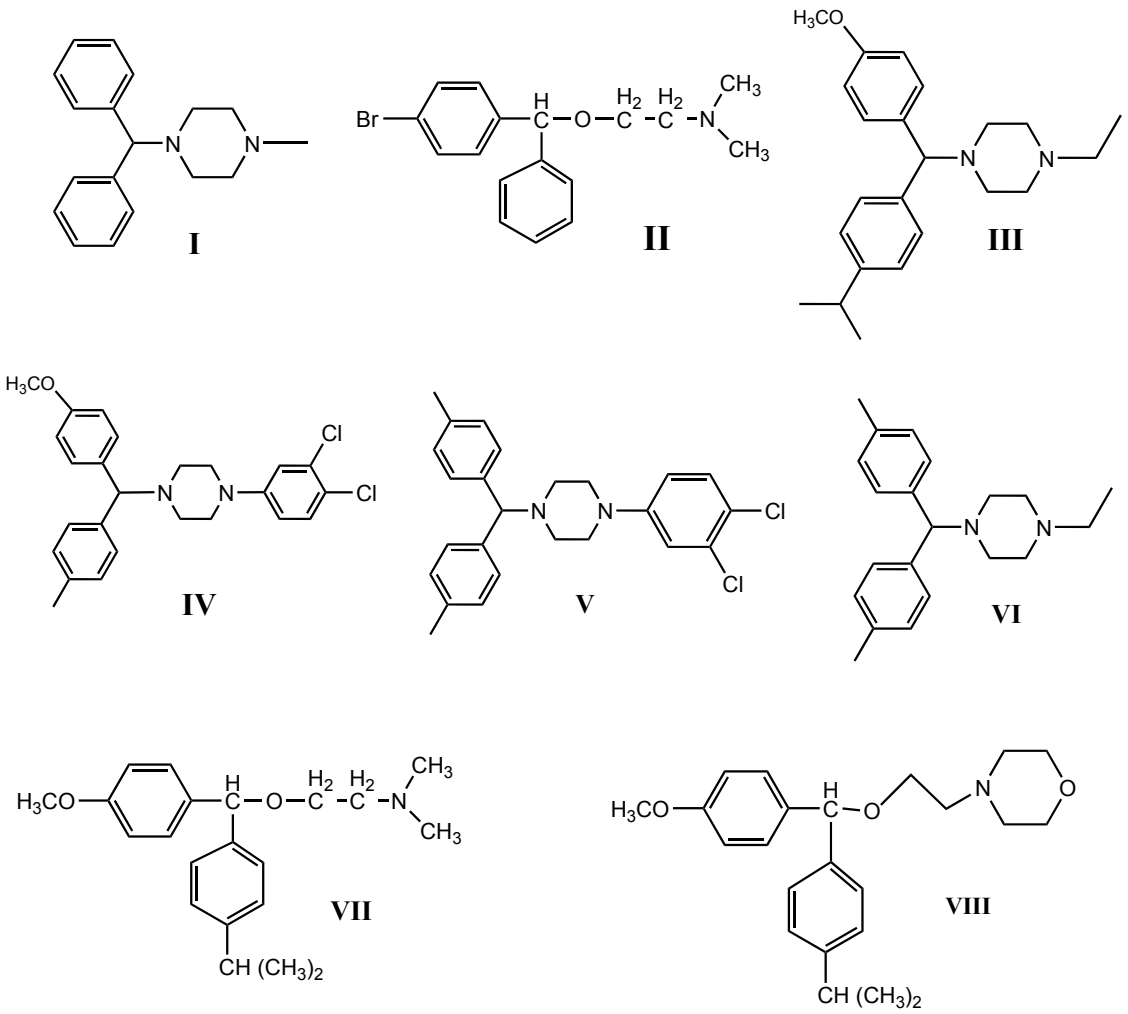
IR (KBr): 2968, 2862, 1651, 1609, 1511, 1463, 1302, 1248, 1172, 1107, 1036, 816 cm^{-1}.

^{1}H N.M.R. ($CDCl_3$) (ppm): 1.04-1.24 (9H, m), 2.3-2.75 (11H, s), 3.73 (3 H, s), 5.24 (1H, s), 6.61-7.03 (8H, m).

^{13}C N.M.R. ($CDCl_3$) (ppm): 13.5, 23.4, 29.7, 45.2, 51, 53.6, 55.5, 72, 112.7, 126.8, 127.9, 128.3, 135.1, 139.8, 148.1, 158.1.

MS: m/z (Regulatory Intensity): 352 (37), 279 (21), 246 (16), 239 (74), 197 (63), 188 (86), 174 (27), 147 (96), 135 (100), 119 (26), 126 (13), 121 (39), 113 (87), 98 (15), 84 (63), 77 (49), 43 (58).

Anal. Calcd. for C_{23} $H_{32}N_2O$: C, 78.36%; H, 9.15%; N, 7.95%. Found: C, 78.44%; H, 9.22%; N, 7.92.



Scheme (1). Structure formulas of Cyclizine (**Cyc**, **I**), Bromodiphenhydramine (Phenyl [*p*-bromophenyl] [dimethylaminoethoxy] methan, **II**), 1-ethyl-4-[(*4*-isopropylphenyl)(*4'*-methoxyphenyl)methyl]-piperazine (**III**, **Cyc-1**), 1-(3,4-dichlorophenyl)-4-[(*4*-tolyl)(*4'*-methoxyphenyl)methyl]-piperazine (**IV**, **Cyc-2**), 1-(3,4-dichlorophenyl)-4-[di(*p*-tolyl)methyl]-piperazine (**V**, **Cyc-3**), 1-ethyl-4-[di(*p*-tolyl)methyl]-piperazine (**VI**, **Cyc-4**), 2-[(*4*-isopropylphenyl)(*4'*-methoxyphenyl)methoxy]-*N*,*N*-dimethylethanamine (**VII**, **Brom-1**), 2-[(*4*-isopropylphenyl)(*4'*-methoxyphenyl)methoxy]-ethylmorpholine (**VIII**, **Brom-2**).

*2.2.4. (***p***-tolyl)(***p***-anisol) methanol (5)*

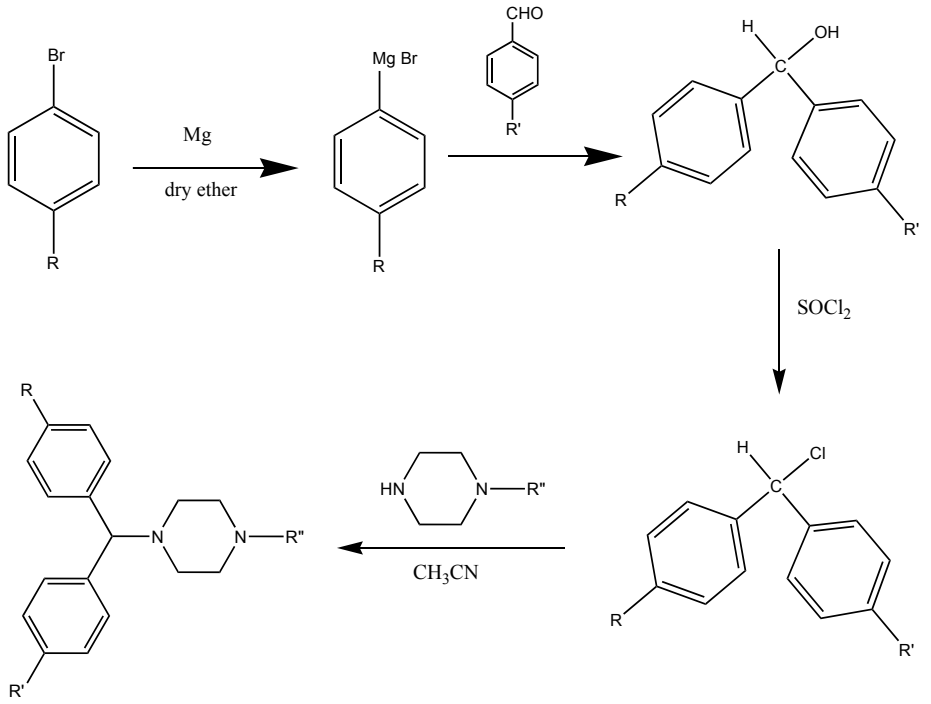
This compound was prepared based on a published method [16]. To a THF (50ml) solution of *p*-methyl benzaldehyde (12g, 0.1mol), anisol magnesium bromide (prepared from 16g *p*-bromo anisol and 2.5g of Mg in 50ml of dry ether) was added dropwise and refluxed for additional 6 days. Then, it was poured into ice-NH_4Cl and the organic layer was separated, washed with brine, re-extracted with diethyl ether, dried over $MgSO_4$ and evaporated under vacuum to obtain the oily compound (8.4 g, 36.84% yield) (Scheme **2**).

2.2.5. Chloro (p-tolyl)(p-anisoll) methan (6)

This compound was prepared based on a published method [15]. $SOCl_2$ (8ml, 0.11mol) was added to a dichloromethane (250ml) solution of alcohol (**3**, 8.4g, 0.04mol). The mixture was refluxed for additional 210 hours and the solvent was evaporated under vacuum to obtain a brown oily compound (4.8g, 52.75% yield) which was used in the next step without further purification (highly sensitive to light) (Scheme **2**).

*2.2.7. 1-(3, 4-dichlorophenyl)-4-[(***p***-anisol)(***p***-tolyl) methyl]-piperazine (Cycl-2) IV*

1-(3, 4-dichlorophenyl) piperazine (23.1g, 0.1mol) was added to an acetonitrile (100ml solution of **6** (4.8g, 0.02mol). The mixture was refluxed for additional 280 hours. Then solvent was removed under vacuum, extracted with diethyl ether, water-washed, re-extracted with 10% H_2SO_4, and neutralized with 10% NaOH. The organic layer was successively water-washed, dried over $MgSO_4$, and evaporated under vacuum to obtain the yellowish oily compound (3.12g, 36.4% yield) (highly sensitive to light) (Scheme **2**). The hydrochloride salt of **IV** was prepared using diethyl ether and HCl.



R	R'	R''	Intermediates and Final Compounds
H	H	CH_3	**1, 2, I**
OCH_3	$CH(CH_3)_2$	CH_3-CH_2	**3, 4, III**
OCH_3	CH_3	$C_6H_5(Cl)_2$	**5, 6, IV**
CH_3	CH_3	$C_6H_5(Cl)_2$	**7, 8, V**
CH_3	CH_3	CH_3-CH_2	**VI**

Scheme (2). Synthesizing Method of intermediates (**1-8**) and final compounds (**I**, **III-VI**).

IR (KBr): 2961, 2871, 1661, 1613, 1513, 1463, 1421, 1364, 1250, 1210, 1038, 841, 755 cm^{-1}.

^1H N.M.R. ($CDCl_3$) (ppm): 2.37 (3H, s), 2.55-2.59 (4H, m), 3.41-3.49 (4H, m), 3.73 (3H, s), 5.26 (1H, s), 6.39-7.06 (11H, m).

^{13}C N.M.R. ($CDCl_3$) (ppm): 24.5, 51.07, 53.6, 55.7, 73.6, 113.8, 114.6, 128.3, 129.3, 129.7, 131.8, 135.1, 135.8, 139.8, 149.1, 158.2.

MS: m/z (Regulatory Intensity): 441 (41), 334 (36), 230 (100), 243 (5), 211 (28), 188 (78), 145 (8), 107 (21), 91 (5).

Anal. Calcd. for $C_{25}H_{26}Cl_2N_2O$: C, 68.03%; H, 5.94%; N, 6.35%. Found: C, 68.09%; H, 5.98%; N, 6.31%.

2.2.8. Di (p-tolyl) methanol (Di [p-methylbenzhydrol] (7)

This compound was prepared from *p*-tolyl magnesium bromide and *p*-methyl benzaldehyde based on a published method [17].

2.2.9. Di (p-tolyl) methyl chloride (Di [p-methylbenzhydryl] chloride) (8)

This compound was prepared from alcohol **(7)** and acetyl bromide based on a published method [16, 17] and used in the next step without further purification.

*2.2.10. 1-(3,4-dichlorophenyl)-4-[di(***p***-tolyl)methyl]-piperazine (V, Cyc-3)*

1-(3, 4-dichlorophenyl) piperazine (23.1g, 0.1mol) was added to an acetonitrile (100ml) solution of **8** (6.3g, 0.023mol). The mixture was refluxed for additional 12 days. Then solvent was removed under vacuum, extracted with diethyl ether, water-washed, re-extracted with 10% H_2SO_4, and neutralized with 10% NaOH. The organic layer was successively water-washed, dried over $MgSO_4$, and evaporated under vacuum to obtain the yellowish oily compound (1.95g, 20% yield) (high sensitive to light) (Scheme **2**). The hydrochloride salt of **V** (oily compound was prepared using diethyl ether and HCl.

IR (KBr): 2983, 2873, 1660, 1587, 1486, 1452, 1404, 1339, 1261, 1154, 1072, 1010, 839, 753 cm^{-1}.

^1H N.M.R. ($CDCl_3$) (ppm): 2.34 (6H, s), 2.55-2.67 (4H, m), 3.41-3.51 (4H, m), 5.35 (1H, s), 6.39-7.05 (11H, m).

^{13}C N.M.R. ($CDCl_3$) (ppm): 24.3, 49.9, 50.2, 72.6, 113.5, 115.6, 122.7, 128.3, 129.7, 131.3, 134.5, 135.1, 139.8, 149.1.

MS: m/z (Regulatory Intensity): 425 (45), 279 (21), 244 (16), 230 (8), 195 (63), 165 (41), 145 (100), 113 (38), 105 (19), 97 (18), 91 (56), 84 (66).

Anal. Calcd. for $C_{25}H_{26}Cl_2N_2$: C, 70.59%; H, 6.16%; N, 6.59%. Found: C, 70.65%; H, 6.23%; N, 6.54%.

*2.2.11. 1-ethyl-4-[di(***p***-tolyl)methyl]-piperazine (VI, Cyc-4)*

1-ethyl piperazine (19ml, 0.1mol) was added to an acetonitrile (100 ml) solution of **8** (2.74g, 0.01mol).). The mixture was refluxed for additional 10 days. Then solvent was removed under vacuum, extracted with diethyl ether, water-washed, re-extracted with 10% H_2SO_4, and neutralized with 10% NaOH. The organic layer was successively water-washed, dried over $MgSO_4$, and evaporated under vacuum to obtain the oily compound (2.3g, 35.4% yield) (highly sensitive to light) (Scheme **2**). The hydrochloride salt of **VI** (oily compound) was prepared using diethyl ether and HCl (highly sensitive to light).

IR (KBr): 2960, 2927, 1645, 1607, 1512, 1455, 1375, 1289, 1162, 1012, 827 cm^{-1}.

^1H N.M.R. ($CDCl_3$) (ppm): 1.04-1.14 (3H, m), 2.27 (6H, s), 2.3-2.44 (8H, s), 5.31 (1H, s), 6.95-7.05 (8H, m).

^{13}C N.M.R. ($CDCl_3$) (ppm): 13.5, 23.4, 49.3, 51.07, 53.6, 73.4, 128.3, 129.3, 135.5, 139.8.

MS: m/z (Regulatory Intensity): 308 (39), 279 (60), 202 (48), 195 (22), 165 (15), 113 (13), 105 (22), 97 (18), 91 (39), 84 (31), 77 (100).

Anal. Calcd. for $C_{21}H_{28}N_2$: C, 81.77%; H, 9.15%; N, 9.08%. Found: C, 82.4%; H, 9.23%; N, 9.02%.

*2.2.8. Phenyl (***p***-bromophenyl) methanol (***p***-bromobenzhydrol) (9)*

This compound was prepared based on a published method [17, 18]. To a diethyl ether (50 ml) solution of *p*-bromobenzaldehyde (1.4g, 0.1mol), phenyl magnesium bromide (prepared from 15.7g bromobenzene and 2.43g of Mg in 50ml of dry ether) was added dropwise and refluxed for 5 hours. Then, it was poured into ice-NH_4Cl and the organic layer was separated, water-washed, re-extracted with diethyl ether, dried over $MgSO_4$ and evaporated under vacuum to obtain a solid compound (m.p: 60-62° C) (Scheme **3**).

*2.2.9. Phenyl (***p***-bromophenyl) methyl bromide (***p***-bromobenzhydryl bromide) (10)*

This compound was prepared based on a published method [17, 18]. A benzene (20 ml) solution of acetyl bromide (11.4g, 0.09mol) was added to a benzene (20 ml) solution of alcohol (**9**, 16g, 0.06 mol). The mixture was refluxed for 11 hours and evaporated under vacuum to obtain an oily brown compound which was used in the next step without further purification.

*2.2.10. Phenyl (***p***-bromophenyl) (dimethylaminoethoxy) methane (Bromodiphenhydramine) II*

This compound was prepared based on a published method [17, 18]. A xylene (20 ml) solution of bromobenzhydryl bromide (**10**, 13g, 0.4mol) was slowly added to a xylene (10 ml) solution of dimethylaminoethanol (7.1g, 0.08mol). Then, the mixture was refluxed for 24 hours, cooled and treated with water, extracted with ether, re-extracted with 10% HCl, neutralized with 10% NaOH, dried over $MgSO_4$ and evaporated under vacuum to obtain the desired oily compound. The hydrochloride salt of **II** (m.p: 143-145° C) was prepared using diethyl ether and HCl and was recrystallized from 2-propanol.

*2.2.13. (***p***-isopropylphenyl)(***p***-anisol) (dimethylaminoethoxy) methane (Bromodiphen-1) (VII)*

A xylene (20ml) solution of Chloro (*p*-isopropylphenyl)(*p*-anisol) methan (**4**, 5.48g, 0.02mol) was slowly added to a xylene (10 ml) solution of dimethylaminoethanol (8ml, 0.1mol). Then, the mixture was refluxed for 9 days, cooled and treated with water, extracted with ether, re-extracted with 10% HCl, neutralized with 10% NaOH, dried over $MgSO_4$ and evaporated under vacuum to obtain the desired oily compound (13.4g, 41% yield).The hydrochloride salt of **VII** was prepared using diethyl ether and HCl and was recrystallized from 2-propanol.

IR (KBr): 2965, 2862, 1651, 1609, 1511, 1463, 1302, 1248, 1172, 1097, 806 cm^{-1}.

^1H N.M.R. ($CDCl_3$) (ppm): 1.31-1.32 (6H, m), 2.31 (6H, s), 2.71-2.9 (2H, m), 3.61-3.67 (3H, m), 3.72 (3 H, s), 5.68 (1 H, s), 6.85-7.3 (8H, m).

^{13}C N.M.R. ($CDCl_3$) (ppm): 24.2, 28.3, 33.8, 54.1, 58.5, 66.5, 83.4, 113.7, 126.5, 127.9, 128.8, 134.5, 139.8, 146.8, 158.9.

MS: m/z (Regulatory Intensity): 328 (11), 255(85), 239 (40), 209 (12), 135 (30), 148 (26), 73 (83), 58 (100), 45 (63).

Anal. Calcd. for $C_{21}H_{29}NO_2$: C, 77.02%; H, 8.93%; N, 4.28%. Found: C, 77.11%; H, 8.98%; N, 4.23%.

*2.2.14. (***p***-isopropylphenyl)(***p***-anisol) (2-morpholinoethoxy) methane (Bromodiphen-2) (VIII)*

A xylene (20ml) solution of Chloro (*p*-isopropylphenyl)(*p*-anisol) methan (**4**, 5.48g, 0.02mol) was slowly added to a xylene (10 ml) solution of 2-morpholinoethanol (11ml, 0.1mol). Then, the mixture was refluxed for 10 days, cooled and treated with water, extracted with ether, re-extracted with 10% HCl, neutralized with 10% NaOH, dried over $MgSO_4$ and evaporated under vacuum to obtain the desired oily compound (7.8g, 21% yield).The hydrochloride salt of **VIII** was prepared using diethyl ether and HCl and was recrystallized from 2-propanol.

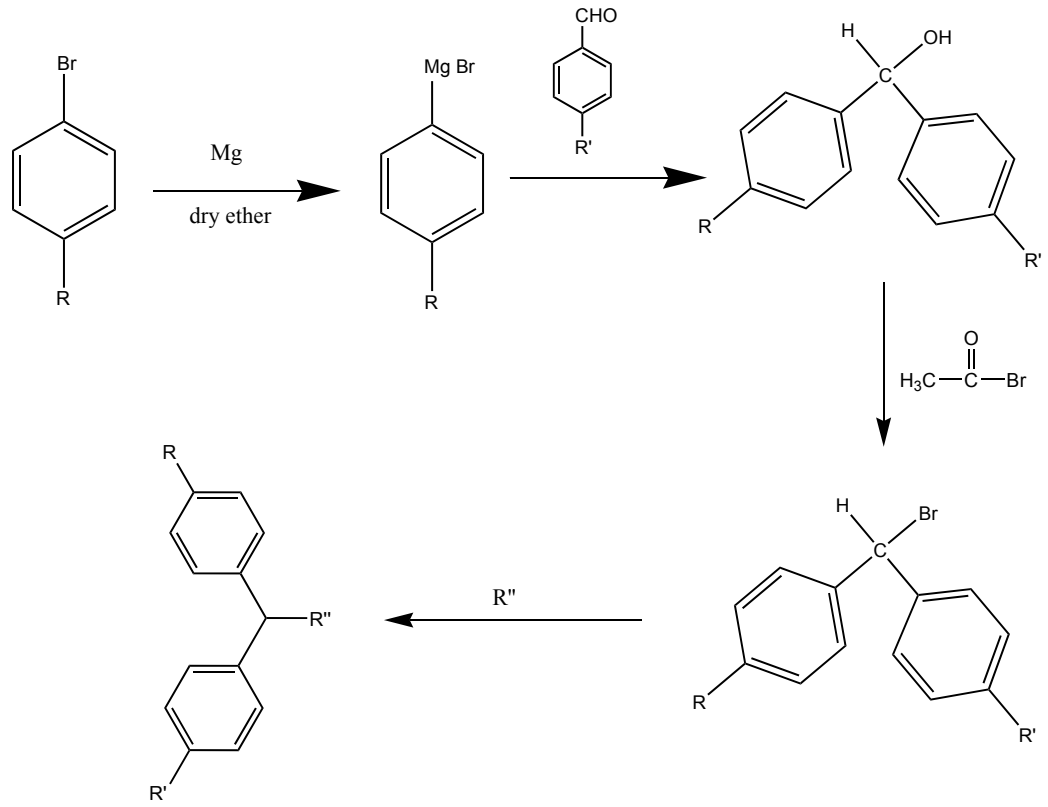
IR (KBr): 2950, 2857, 1652, 1610, 1514, 1455, 1302, 1247, 1172, 1118, 1035, 825 cm^{-1}.

^1H N.M.R. ($CDCl_3$) (ppm): 1.17-1.31 (6H, m), 2.51-2.87 (6H, s), 3.5-3.72 (7H, m), 3.78 (3 H, s), 5.3 (1 H, s), 6.84-7.3 (8H, m).

^{13}C N.M.R. ($CDCl_3$) (ppm): 24, 29.7, 33.7, 54, 55.2, 58.3, 66.5, 66.9, 83.4, 113.8, 126.8, 128.2, 129, 130.1, 139.7, 146.8, 158.8.

MS: m/z (Regulatory Intensity): 370 (52), 255 (4), 239 (88), 115 (15), 100 (100), 87 (31), 42 (7).

Anal. Calcd. for $C_{23}H_{31}NO_3$: C, 74.76%; H, 8.46%; N, 3.79%. Found: C, 74.82%; H, 8.51%; N, 3.74%.



R	R'	R''	Intermediates and Final Compounds
H	Br	Dimethylamino ethanol	**9, 10, II**
OCH_3	CH $(CH_3)_2$	Dimethylamino ethanol	**VII**
OCH_3	CH $(CH_3)_2$	2-morpholinoethanol	**VIII**

Scheme (3). Synthesizing Method of intermediates (**9**, **10**) and final compounds (**II**, **VII** and **VIII**).

2.3. Pharmacological Methods

2.3.1. Animals

Adult male wistar rats (Pasteur Institute, Tehran), weighing 250-300g at the start of the experiment, were randomly housed in groups of three to four per cage, in a temperature-controlled colony room, under 12h light/dark cycle. Animals were given free access to water and standard laboratory rat chow (Pars Company, Tehran, Iran). All the experiments were implemented between 11 a.m. to 4 p.m. under normal room light and 25°C. This study was carried out in accordance with the policies provided in the Guide for the Care and Use of Laboratory Animals (NIH) and those by the Research Council of Shahed University of Medical Sciences, Tehran, Iran.

2.3.2. Anti-Inflammatory Activity

2.3.2.1. Acute Inflammation

2.3.2.1.1. Formalin-induced rat paw edema

The rats (n =72) were divided to control (n = 8) and eight treatment groups (drug I-VIII, n = 8 in every group test). To the treatment groups, the drugs (17 mg/kg, i.p) were given 30 minutes prior to formalin injection (50µl of 3%) in the right hind paw subplantar surface. The superior-inferior paw diameter was measured before (zero time) and at 0.5,1, 2 and 3h after the formalin injection by using a caliper [11]. The differences in paw diameters between control and treatment groups were collected as the required data and subjected to statistical analysis.

2.3.2.1.2. Histamine-induced rat paw edema

The animals were treated in a manner similar to those in formalin-induced paw edema models. Only histamine was applied at dose of 300µg in 100µl. [12].

2.3.2.2. Chronic Inflammation

Cotton pellet-induced granuloma formation

The cotton pellets-induced granuloma in rats was studied according to the method of D'Arcy *et al.* (1960) [13]. The control and treatment animals were anaesthetized with ketamine injection (100 mg/kg; i.p) and sterile cotton pellets weighing 30±1mg were implanted subcutaneously into both sides of the groin region of each rat. The animals in all groups received the vehicle (saline) or drugs for seven consecutive days since the day of cotton pellet implantation.

On the 8th day, the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and dried in an oven at 60°C for 24 hours to constant weight before the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation.

3. RESULTS

3.1. Chemistry

Cyclizine **(I)**, Bromodiphenhydramine (**II**) and their new analogues (**III-VIII**) were synthesized by reaction of substituted benzhydryl chlorides (**2**, **4**, **6** and **8**) and piperazine compounds for producing **I** and **III-VI**, or substituted aminoethanol compounds for producing **II**, **VII** and **VIII**.

Following our previous experiments, methyl, methoxy and isopropyl groups on the aromatic rings had high electron donating characters that induced more electron density and dipole moments on the molecules which could produce more pharmacological properties [15, 17, 19-21]. Also, replacing dimethylamine group by morpholine with many pharmacological properties [22-25], and methyl piperazine by ethyl and 3, 4-dichlorophenyl piperazines could produce more anti-inflammatory activities [15, 17] on our new synthesized drugs (**III-VIII**).

Spectroscopic data (IR, ^{1}H and ^{13}C NMR, Mass) confirmed the structure of new compounds. The melting points of the known compounds also confirmed their identity. The purity of each compound was checked by TLC using ethyl acetate-hexane as the eluent.

3.2. Pharmacology

3.2.1. General Consideration

Mortality (number of death), morbidity (defined as any abnormal condition or behavior due to a disorder), irritability (a condition of aggressiveness or increased response on handling) and other related abnormal states were observed in experimental animals. However, the motor coordination index (measured by Rota-rod apparatus, Harvard, UK) did not indicate any significant differences between treated rats.

3.2.2. Acute Inflammation

3.2.2.1. Histamine-induced rat paw edema in cyclizine derivatives

The anti-inflammatory effects of **I** and **III-VI** against acute paw edema induced by phlogistic agent histamine is shown in (Fig. **1A**). All drugs caused significant anti-inflammatory effects 33, 39, 36, 39 and 39 % respectively within 1 hour after histamine injection ($P< 0.01$ and 0.05) comparing to the control group. Also, newly synthesized drugs (**III-VI**) remarkably decreased inflammation in 2 or 3 hours after histamine injection comparing to **I**.

3.2.2.2. Histamine-induced rat paw edema in bromo diphenhydramine derivatives

The anti-inflammatory effects of **II**, **VII** and **VIII** against acute paw edema induced by phlogistic agent histamine are shown in (Fig. **1B**). An hour after histamine injection, compounds **II**, **VII** and **VIII** generated more significant anti-inflammatory effects 38, 41 and 43 %, respectively, comparing to the control group. However, these effects were omitted in 2 or 3 hours due to histamine injection in all drugs.

3.2.2.3. Formalin-induced rat paw edema in cyclizine derivatives

The anti-inflammatory activities of **I** and **III-VI** drugs were measured at the dose of 17mg/kg against acute paw edema induced by formalin which is demonstrated in (Fig. **2A**). All drugs showed significant anti-inflammatory effects 34, 33, 36, 39 and 39 % respectively within 1 hour after histamine injection, comparing to the control animals. Noticeably, compounds **V** and **VI** could remarkably decrease inflammation in 2 or 3 hours after formalin injection comparing to the control group.

3.2.2.4. Formalin-induced rat paw edema in bromodiphenhydramine derivatives

The **II**, **VII** and **VIII** anti-inflammatory effects at dose of 17mg/kg against acute paw edema induced by formalin is shown in (Fig. **2B**). As indicated, drugs **VII** and **VIII** could produce significant ($P<0.05$) anti-inflammatory activities 38 and 41% respectively 1 hour after formalin injection. However, these effects were less potent in control and drug II animal groups. Also in 2 and 3h following histamine administration, no significant effects were shown for any drugs.

3.2.3. Chronic Inflammation

3.2.3.1. Cotton pellet-induced granuloma formation in cyclizine derivatives in rats

The effects of **I** and **III-VI** on cotton pellet-induced granuloma formation are shown in (Fig. **3A**). The weight gain of cotton pellet from 67.10 ± 11.58g in control rats reached to 85.10±22.34g, 94.14±16.07g and 74.20±4.26g for drugs **I**, **II** and **III** respectively. However, statistical analysis did not show any significant differences between groups**.**

3.2.3.2. Cotton pellet-induced granuloma formation in bromodiphenhydramine derivatives in rats

In (Fig. **3B**) the effects of compounds **II**, **VII** and **VIII** on cotton pellet-induced granuloma formation are shown. As indicated, there were no significant differences between control cotton pellet weight (67.10±11.58g) and compound **II** (76.20±18.24 g), **VII (**73.17±14.47 g) and **VIII (**70.95±9.26g).

4. DISCUSSION

Compounds with H_1-antihistaminic activity might be applied as a useful therapeutic in treatment of various allergic and inflammatory conditions due to histamine release [26]. Cyclizine (**I**) is a famous drug of piperazine derivatives [27] and bromodiphenhydramine **(II)** is a relatively low molecular weight with high lipid solubility and a famous drug of ethanolamine derivatives which belong to the H_1-antihistamine group of drugs [28].

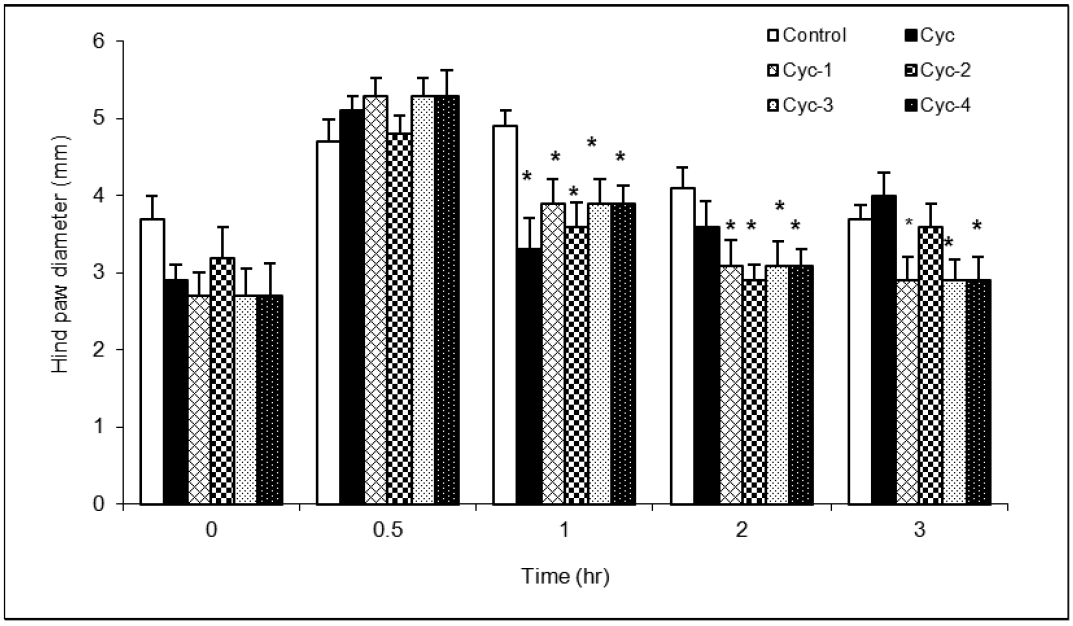
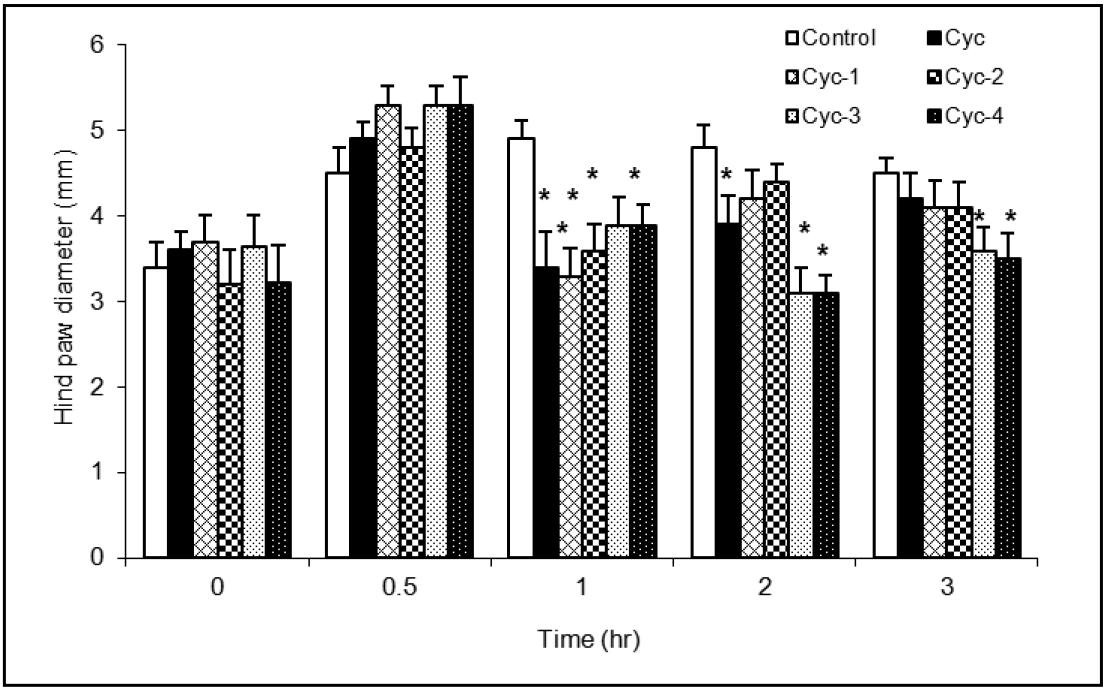


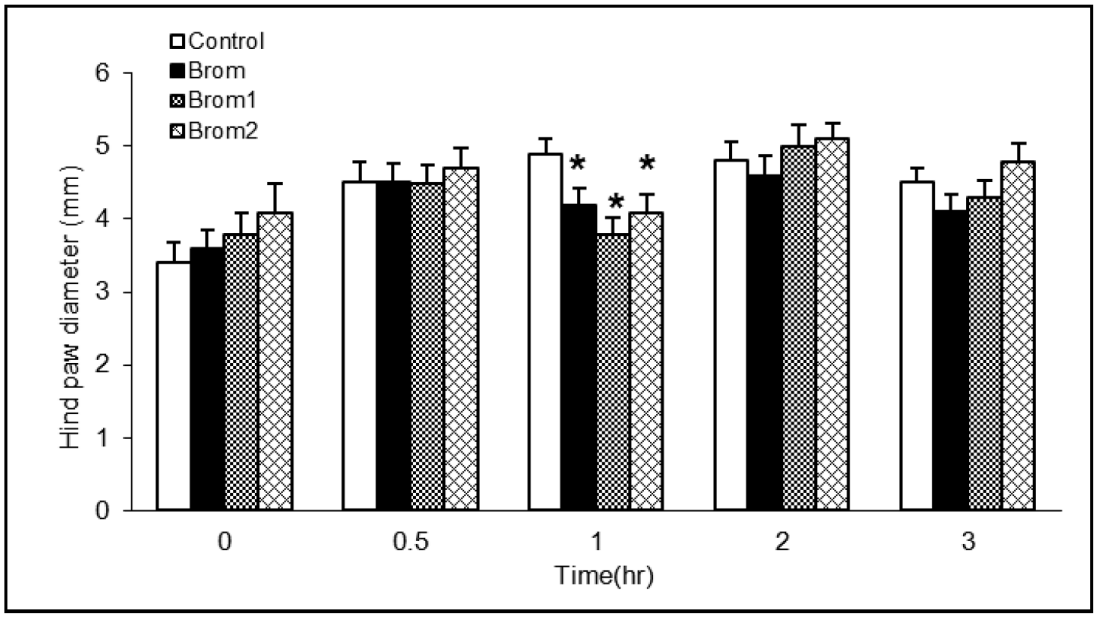
Fig. (1). Anti-inflammatory effects of **I**, **III-VI** (**A**) and **II**, **VII**, **VIII** (**B**) in histamine-induced rat paw edema. Edema was measured in 0, 0.5, 1, 2 and 3 h after histamine injection. Bars show mean ± SEM of paw diameter. *, ** respectively show P <0.05, 0.01 compared with control. (n = 12).

In this study, some new derivatives of **I** and **II** inducing changes in substitutions on phenyl and amine moieties were synthesized and acute and chronic anti-inflammatory effects of these new compounds were evaluated by standard pharmacological tests [11-13].

The results showed that acute anti-inflammation effects of all drugs were started 60 minutes after histamine or formalin injection but these effects continued for longer period of time (2 and 3 hours after histamine or formalin application) only for some new compounds (**III-VI** for histamine application and **V-VI** for formalin application) which might be due to more half-life of these new drugs comparing to **I** and control groups. Also, for compounds **VII** and **VIII**, these effects were seen only 1 hour after histamine or formalin injection while these compounds could remarkably decrease inflammation comparing to control group, similar to **II**.



A

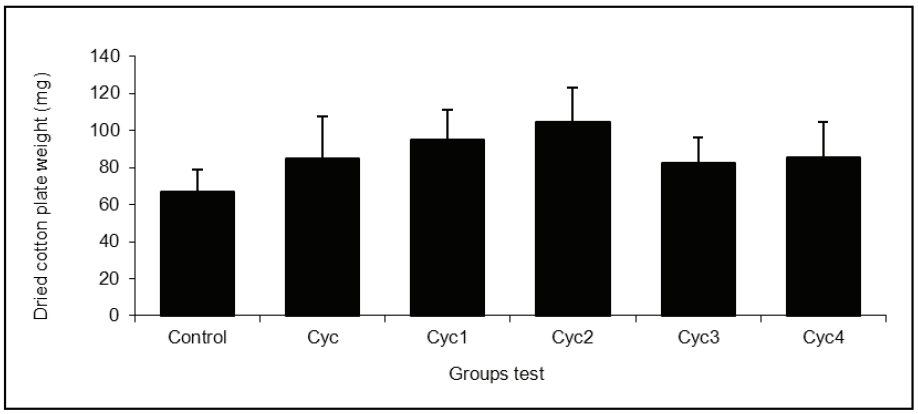


B

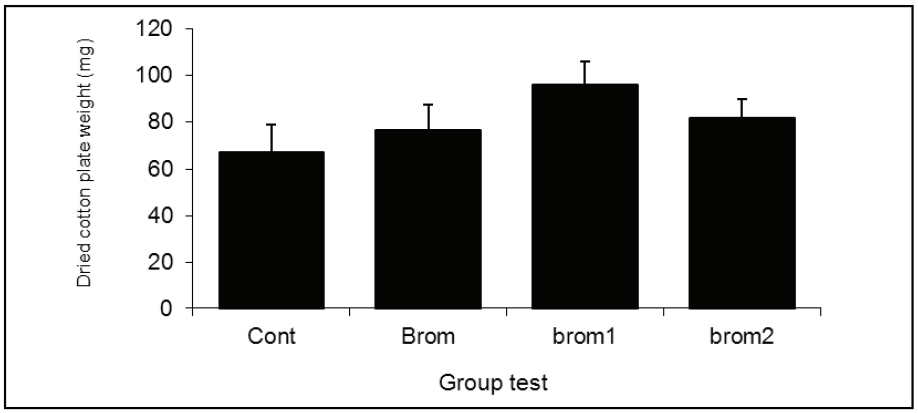
Fig. (2). Anti-inflammatory effects of **I**, **III-VI** (**A**) and **II**, **VII**, **VIII** (**B**) in formalin-induced rat paw edema. Edema was measured in 0, 0.5, 1, 2 and 3 h after formalin injection. Bars show mean ± SEM of paw diameter. * P <0.05 compared with control. (n = 12).

Also, similar results for **V** and **VI** in histamine or formalin rat paw edema proved that adding ethyl or 3, 4-dichlorophenyl to the right side of piperazine moiety had the same effects on acute inflammation to conclude that this side of the molecule would have a little role in changing anti-inflammatory properties in this family.

Comparing to **II**, the lower acute anti-inflammatory effects of **VII** proved that adding methoxy or isopropyl groups to phenyl rings of **II** could not produce better effects on decreasing inflammation unlike methyl groups in these moieties [16] which might be due to higher steric hindrance of these groups. The lower effects of **VIII** comparing to **VII**,



A



B

Fig. (3). Chronic anti-inflammatory effects of **I**, **III-VI** (**A**) and **II**, **VII**, **VIII** (**B**) in cotton plate implantation model. There was no significant effect between control and treatment animals. Bars show mean ± SEM cotton dry weight (n= 12).

proved the better results by dimethylamine comparing to morpholine groups which might be due to higher electron donating by two methyl groups producing more activated amine moiety in this side of the molecule (1 hour after histamine or formalin application).

The results from the cotton pellet-induced granuloma formation in rats, however, showed that none of the drugs (**I-VIII**) were effective enough to reduce the reactions and intermediates of chronic inflammation which might be due to complexity of chronic inflammation mechanism(s) and its immunity against (only) histamine release. Nevertheless, it must be reminded that anti-inflammatory activities of these new drugs in both formalin and histamine injection tests showed that they must act more through other unknown mechanism(s) than preventing histamine release.

5. CONCLUSION

It can be concluded that substitution of phenyl by tolyl, anisol and cumene groups in piperazine family can remarkably decrease inflammatory activities in these new drugs. However, the results proved that these effects were stronger and would remain for a longer period (2 and 3 hours after histamine or formalin injection) in compounds **V** and **VI** which might be caused by lower steric hindrance of tolyl group comparing to others (phenyl, anisol and cumene). Also for compounds **VII** and **VIII**, the results showed that substitution of dimethylamine by morpholine group could not decrease inflammatory activities of these new drugs which might be caused by higher electron donating of two methyl groups in dimethylamine comparing to morpholine that produced more activated amine moiety in this side of the molecule (1 hour after histamine or formalin application) in ethanolamine family. It seemed that the acceptable performance of the new drugs might be related to their more antagonistic effects on H_1 histamine receptors.

CONFLICT OF INTEREST

This research is not a part of our normal lecturing, employment, consultation, and involvement; and no institution will require any rights from this work.

In addition, no patent has been applied nor any commercial right has been given to any company and/or institution, and it will not be done later either.

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